Phenotypic Plasticity of Locomotion Performance in the Seed Harvester *Messor capensis* (Formicidae)

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ABSTRACT

Temperature dependence, energetics, and speed of locomotion have important implications for the ecology and evolution of ants. Here, we report the results of a full-factorial study investigating the responses of active metabolic rate (AMR), voluntary locomotion speed, and cost of transport (COT) to three temperature acclimations (7–10 d; 20°, 25°, and 30°C) and three test temperature treatments (2 h; 20°, 25°, and 30°C) in a seed-harvester ant, *Messor capensis*. By using a strong-inference approach, we assessed these trait responses in the context of the beneficial acclimation hypothesis and its alternatives. Results showed that AMR was not affected by acclimation temperature, indicating limited phenotypic plasticity for this trait. By contrast, voluntary running speed was consistently higher when ants were acclimated at 25°C, providing support for the optimal acclimation hypothesis. COT was not affected by acclimation or treatment temperature. In addition, while AMR was repeatable and similar across temperatures ($r = 0.371–0.683$), the repeatabilities of running speed ($r = 0.191–0.826$) and COT ($r = 0.166–0.903$) were highly variable, suggesting constraints on ant locomotion under certain thermal conditions. Minimum COT (298 J kg^{-1} m^{-1}) in *M. capensis* was well within the range of values for other terrestrial arthropods of a similar body size. This study emphasizes the need to investigate a variety of performance traits rather than a single one and to expand the limited body of work on plasticity of insect locomotion.

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Introduction

In most animals, locomotion is central for sustaining life. It influences territory vigilance, mating and foraging success, thermoregulation, dispersal, and escape from predators. In ectotherms, locomotion performance is temperature dependent, as are a range of other whole-organism physiological traits (Chown and Nicolson 2004; Angilletta 2009). Outside of an optimal temperature range, locomotion is progressively impaired, potentially affecting the fitness of the organism (Hertz et al. 1983; Bennett 1990). Because most ectotherms experience different scales of environmental heterogeneity (daily and seasonal temperature fluctuations, fine- vs. coarse-grained habitat scales; Levins 1968; Bennett 1990), several theoretical models and hypotheses predict the extent to which performance capacity will respond to these changes over both the short term (via plasticity) and the long term (via evolution of both trait means and plasticity; e.g., Van Tienderen 1991; Gabriel and Lynch 1992; Zamudio et al. 1995; Kingsolver and Huey 1998; de Jong 1999). The beneficial acclimation hypothesis (BAH) is one of the most prominent of these. It predicts that performance will be enhanced for individuals measured at a temperature similar to the acclimation temperature to which they have previously been exposed, relative to that of individuals acclimated to other temperatures (Levins 1969; Leroi et al. 1994). Several alternative hypotheses have been proposed. Warmer- (or colder-) is-better (WIB or CIB) predicts that individuals acclimated to warm (or cold) temperatures will have higher performance across all temperatures relative to organisms acclimated to colder (or warmer) temperatures (Zamudio et al. 1995; Huey and Berrigan 1996). The optimal acclimation hypothesis (OAH) predicts that individuals acclimated to intermediate temperatures will have higher performance across all temperatures than individuals acclimated to higher or lower temperatures (Cohet and David 1978; Huey and Berrigan 1996). Despite the attention that the BAH has received for a variety of performance traits (e.g., Leroi et al. 1994; Stillwell and Fox 2005; Geister and Fischer 2007; Marais and Chown 2008) and its biological significance (Huey et al. 1999; Hollander 2008; Angilletta 2009), a relatively small proportion of studies have focused on locomotion performance.

The thermal acclimation of locomotion performance and its underlying biochemical and molecular mechanisms have been well explored in studies of fish and marine invertebrates (for reviews, see Johnston and Temple 2002; Pörtner et al. 2006) and, to a lesser degree, in studies of amphibians and reptiles (e.g., Kaufmann and Bennett 1989; Wilson et al. 2007). In general, burst, sustained swimming or running, and jumping performance are modified by acclimation, but the direction of

the modification does not always follow predictions of the BAH. For example, out of 15 studies examining the effects of acclimation on fish swimming performance, six found support for the BAH, seven found partial support, and two were unsupportive (Angilletta 2009). Moreover, amphibians in larval stages seem to show more acclimatory capacity than adults, a pattern that may reflect the magnitude or predictability of temperature fluctuations in their respective environments (Knowles and Weigl 1990; Wilson and Franklin 1999, 2000). By contrast, the limited number of data on the thermal acclimation capacity of insect locomotion performance hinders such a comparative approach, despite the potentially critical role of locomotion in enhancing fitness, in at least some insect groups (e.g., obligatory host-searching behavior for parasitoids, mating success in flies; Partridge et al. 1987; Bigler et al. 1988). Indeed, so far as can be ascertained, only eight studies have examined the effects of within-generation thermal acclimation on locomotion performance of insects (physical traits such as walking speed, ability to reach resources, and takeoff flight). Of these, only one incorporated adult acclimation (i.e., reversible responses; Kristensen et al. 2008), five focused on developmental plasticity (i.e., rearing temperature; Barnes and Laurie-Ahlberg 1986: with acclimation hypotheses also tested by Crill et al. 1996; Huey et al. 1999; Gibert et al. 2001; Frazier et al. 2008), and two included short-term hardening (heat hardening; Thompson et al. 2001; Loeschcke and Hoffmann 2007). These studies have produced mixed support for the BAH. For example, Kristensen et al. (2008) found that under cold field conditions, adult cold-acclimated flies (*Drosophila melanogaster*) performed better (measured by numbers reaching bait stations) than warm-acclimated flies but showed substantial costs under warm conditions, thus supporting the BAH. By contrast, both Crill et al. (1996) and Gibert et al. (2001) found that although developmental temperature had a significant effect on walking speed of *D. melanogaster*, their specific outcomes provided support for the optimal developmental temperature hypothesis (similar to the OAH). The effects of acclimation on the metabolic costs of locomotion have received even less attention. Berrigan and Partridge (1997) found that individuals reared (but also kept for several generations) at lower temperatures tended to have higher active metabolic rates (AMRs) than those reared at warmer temperatures when tested under the same temperature treatment.

Given the limited taxonomic coverage across all of these studies (all but one utilized *D. melanogaster*) and the significance of locomotion and its costs as major fitness components, additional investigations are required to establish the extent and form of plasticity in these traits in insects (for other groups, see, e.g., Li and Wang 2005; Deere and Chown 2006). Ants provide excellent model organisms for studies of insect locomotion and its phenotypic plasticity for two major reasons. First, they are central-place foragers, and therefore locomotion is an important contributing factor to their success in various environments (Hölldobler and Wilson 1990). Indeed, the energetic cost and speed of locomotion have important implications for the fitness of foraging ants and contribute to the overall energy budget of whole ant colonies (Nielsen and Baroni-Urbani 1990; Lighton et al. 1993). Second, a large body of work has explored the relationships between locomotion costs and speed, foraging strategies, and the variation of these traits with body morphology and temperature, providing substantial background information for investigations of the plasticity of locomotion and its costs (e.g., Shapley 1920, 1924; Nielsen et al. 1982; Bartholomew et al. 1988; Duncan and Crewe 1993; Duncan and Lighton 1994; Lighton and Duncan 2002; Schilman and Roces 2005). In general, within the range of optimal temperatures, ant AMR increases with walking speed, walking speed increases with temperature, and the minimum cost of transport (MCOT, defined as the slope of the relation between walking speed and metabolic rate; Lighton et al. 1993) is independent of temperature (e.g., Lighton and Feener 1989; Weier et al. 1995; Lighton and Duncan 2002; Hurlbert et al. 2008). Moreover, interspecific comparisons have assessed the extent to which ants and other insects support the purported general relationship of mass and MCOT derived from investigations of vertebrates (see discussion in Lighton and Duncan 2002).

In this study, we therefore investigate the plasticity of locomotion and its costs in *Messor capensis*, a seed-harvester ant endemic to South Africa. Specifically, we ask whether the effects of temperature acclimation on running speed, AMR, and COT are consistent with the BAH or with competing hypotheses (WIB, CIB, OAH, and no acclimation [NA]), using an a priori strong-inference approach (see Huey et al. 1999; Deere and Chown 2006). For all hypotheses, we explicitly assume that high running speed and low AMR and COT represent the situation of greatest fitness (i.e., foraging rate is high, but its costs are low). NA is taken as the null situation, with the acknowledgment that specific, unpredictable situations may also lead to NA (see Deere and Chown 2006). We also investigate the repeatability of locomotion speed and associated energetic costs to assess whether the individual variation of ant locomotion performance is consistent over trials (Bennett 1980, 1987; Boake 1989; Chown 2001) and to determine whether there are distinct repeatability differences across acclimation- and test-temperature conditions. Repeatability of a trait (i.e., consistent among-individual differences) is one of the prerequisites for a response to natural selection (see discussion in Endler 1986).

Material and Methods

Species Description and Collection

Messor capensis is a relatively common seed-harvester ant in the semiarid and arid areas of the southwestern region of South Africa and is considered a major seed forager in most areas where it occurs (Kerley 1991; Milton and Dean 1993). In February 2008, several hundred worker ants of similar size and shape (given species polymorphism) were collected from foraging trails surrounding nest mounds at Bartholomeus Klip farm (33.736°S; 19.038°E; Western Cape Province, South Africa) and transported to the laboratory on the same day in

insulated containers. Workers of similar size were selected because of their significance for central-place foraging and to reduce any confounding effects associated with size or caste.

Acclimation Experiments

In advance of acclimation trials, the critical thermal limits of the species were established on a separately collected set of individuals to ensure that acclimation temperatures were well removed from these limits. Field-collected ants were held for 3 d in the laboratory at 25°C with access to water. Critical thermal maxima (CT_{max}) and minima (CT_{min}) were assessed in these individuals with dynamic heating and cooling protocols, respectively (following methods used by Jumbam et al. 2008). Ants were loaded individually into 10 tubes, and an eleventh tube was used to monitor chamber temperature in a double-jacketed Perspex "organ pipe" system connected to a programmable water bath (LTC-12, Grant Instruments). For CT_{max}, ants were initially held for 30 min at the starting temperature of 35°C, to allow equilibration to these conditions. The program then ramped temperature upward at 0.5°C min^{-1}. The temperature at which muscle spasms and loss of righting ability occurred was defined as CT_{max} and was noted for each individual. For CT_{min}, a starting temperature of 15°C was used, with a 30-min equilibration period. Thereafter, the temperature was ramped downward at 0.5°C min^{-1} until the loss of coordinated muscle function occurred when the ant was prodded mildly with a fine paintbrush.

For the main acclimation trial, ants were randomly divided into three groups, each kept in a plastic box (27 cm × 27 cm × 15 cm) containing soil and seeds from their natural environment and water and additional food (oats and seeds) ad lib. Each group was assigned to one of three acclimation temperatures (ACCs; 20°C, 25°C, and 30°C) maintained by temperature-controlled chambers (Labcon, Johannesburg) for 7–10 d. It is well established that 5–7 d is sufficient for complete thermal acclimation of several physiological traits in other insect species (e.g., Hoffmann and Watson 1993; Terblanche et al. 2006). The ACCs selected were well within the critical limits of the species (see "Results") and well within the range of environmental temperatures (soil surface) documented at a nearby location in the field (Fig. 1), minimizing potentially confounding effects due to stressful temperature treatments (Huey et al. 1999; Wilson and Franklin 2002; Woods and Harrison 2002). Mean (±SE) hourly temperature inside the three ACC chambers, obtained from thermochron iButtons (model DS1921), were 20.19° ± 0.01°C, 24.72° ± 0.01°C, and 30° ± 0.01°C. A photoperiod of 12L : 12D was used, corresponding to field conditions at the time of collection. Food was removed 24 h before the experiments started.

Ant running speed and metabolic cost were measured in a "running-tube respirometry" system (Lighton and Feener 1989) at each of the three test temperatures (TTs; 20°, 25°, and 30°C) until all three ACC groups had been run at all possible TTs. The order of acclimation and temperature groups used throughout the day was randomized to avoid diurnal effects.

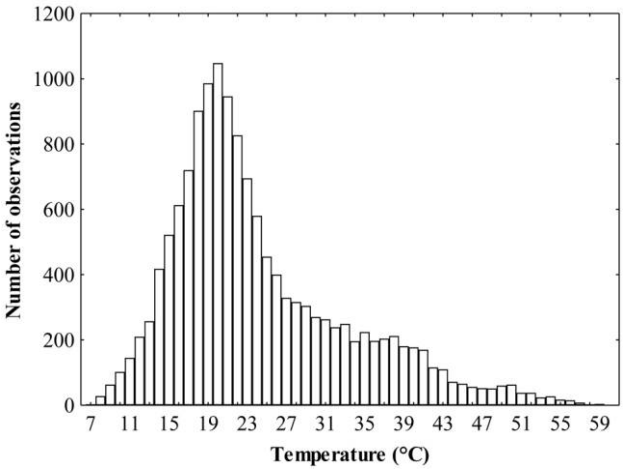

Figure 1: Frequency distribution of soil temperatures measured 1 cm below the sand surface at a field location near the field site (Hawekwas: 33.6741°S, 19.0584°E) during ant activity season, September–March. The histogram uses data from 2006–2009.

In total, 15 individuals were used per ACC-TT group, and individuals were not reused after an ACC-TT trial.

Respirometry

The standard running-tube respirometry technique provided simultaneous assessment of running speed and CO_2 production by ants (for detailed methods, see Lighton and Feener 1989; Lighton et al. 1993; Lighton and Duncan 2002). The system consisted of a glass cuvette 66 cm in length and 1.5 cm in diameter (Sable Systems, Las Vegas, NV; air replacement time <30 s) incorporated into a LI-7000 infrared gas analyzer (LiCor, Lincoln, NE) flow-through respirometry system; the cuvette was equipped with 64 optical gates that sensed motion when the optical beam was broken. Compressed air (21% O_2, N_2 balanced) scrubbed of CO_2 (with soda lime) and water (with silica gel and Drierite) was pushed through the cuvette at a flow rate of 200 mL min^{-1} (at STPD) with a mass flow controller (Sidetrak, Sierra Instruments, Monterey, CA). Ant body length ranged from ~5 to 10 mm. Tubing connecting the running cuvette to the analyzer was kept as short as possible to improve the analyzer's response time, which varied from 1 to 25 s, depending on the ant's position inside the running tube. A thermocouple connected to a Sable Systems TC-1000 meter was used to monitor temperature inside the cuvette during the experiment. The whole respirometry setup was positioned inside a climate-controlled chamber (±0.4°C; Labcon). Temperature, ant CO_2 production, and ant position in the running tube were continuously recorded at 1-s intervals with EXPEDATA (Sable Systems International data acquisition software; a typical recording is given in Fig. 2). Baseline readings for the empty running tube were taken before and after each individual trial, which lasted ~2 h. Ant body mass was recorded before each trial and used in the analyses. Running tubes were cleaned with ethanol before each trial in order to reduce con-

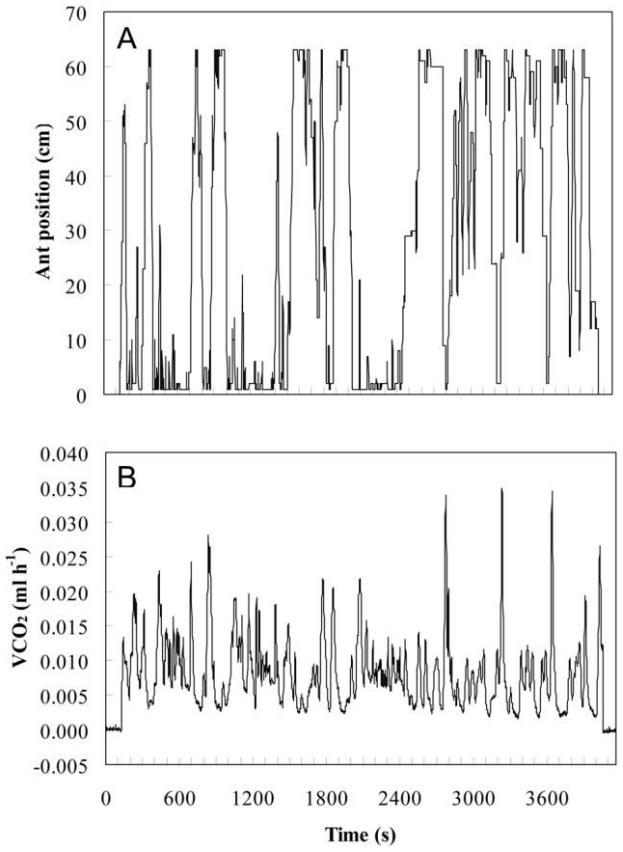

Figure 2: Example of a typical recording with the position of the ant in the running tube (*A*) and the carbon dioxide production during locomotion (*B*) for a single individual of *Messor capensis* acclimated at 30°C and tested at 25°C.

founding effects of ant pheromones potentially altering the behavior of subsequent individuals.

Analyses

Ant displacement data were obtained from the absolute cumulative difference between subsequent positions of an individual ant that moved voluntarily in the tube (without prodding) and converted into running speed. For each individual trial, all intervals of constant displacement versus time with a coefficient of determination (r^2) of >0.95 were selected (following methods of Lighton et al. 1993). Only periods greater than 1 min were used, to ensure stable CO_2 production measurements, resulting in two to six intervals of 1–6 min per individual trial. Of 135 ants tested, 14 did not walk consistently for more than 1 min and therefore were not included in the analyses. The average speed in centimeters per second over each selected interval was determined from the slope of the regression between displacement (cm) and time (s), given that the displacement is 0 at t_0. The ant's mean active CO_2 production (mL CO_2 h^{-1}) was determined from the same time intervals. Mean AMR corrected to STP and mean running speed were obtained by averaging all repeated recordings per individual trial. To follow previous standard determinations of COT and MCOT (Lighton et al. 1993), AMR was converted to mass-specific AMR in watts per kilogram, with a respiratory quotient of 0.84 (as found for active and nonactive *Messor pergandei*; Lighton and Duncan 2002). Potential small Doppler effects (see Berrigan and Lighton 1994; Lipp et al. 2005) were not accounted for in the AMR determinations. However, because the methods were consistent across ACC and TT groups and the trial periods were sufficiently long (~2 h) to gather multiple >1-min CO_2 production readings within single runs, these small effects should not affect the analyses. Because it was possible that we did not have enough intraindividual AMR and speed data for an accurate determination of individual slopes (MCOT), we chose to determine COT (J kg^{-1} m^{-1}), which is calculated as mass-specific AMR (W kg^{-1})/running speed (m s^{-1}; Lighton et al. 1993). With COT, both repeatability and the acclimation hypotheses could be assessed.

A 3 × 3, full-factorial design (following Stillwell and Fox 2005) was used to test the effects of ACC and TT treatments on AMR, running speed, and COT by means of ordered-factor orthogonal polynomial contrast analyses implemented in SAS (ver. 9.1, SAS Institute, Cary, NC). The 3 × 3 design is critical for assessing the rank order of treatments and thus acclimation hypotheses. The five hypotheses (BAH, WIB, CIB, OAH, and NA) were evaluated by examining the strength and direction of the main effects, along with the single-degree-of-freedom contrasts for ordered factors with three levels (linear and quadratic effects; Crawley 2007; reviewed in Huey et al. 1999). The predictions of linear and quadratic effects for each hypothesis, under the assumption that high running speed and low AMR and COT enhance performance and fitness, are provided in Table 1. Because of the balanced design requirement of orthogonal polynomial contrasts (Sokal and Rohlf 1995), the sample size across all ACC-TT groups was reduced to 12 individuals, which was the lowest sample size for a treatment combination, given that some ants did not run consistently in the tube (see above). Individuals were deleted randomly by customized software. Mean AMR and running speed were $\log_{10}$ transformed to normalize distributions before these analyses. In all traits investigated, the data met the major assumptions of this approach (balanced design, homogeneity of variances, and normally distributed data residuals; Huey et al. 1999). Repeatability (r) was calculated as the intraclass correlation coefficient (Falconer and Mackay 1996) after ANOVAs (Lessells and Boag 1987). Repeatability was determined for AMR, running speed, and COT by the use of multiple >1-min intervals of constant displacement within single 2-h temperature trials (i.e., two to six repeated measurements per individual). Ideally, separate trials would have been more favorable for calculating repeatability, but pilot trials revealed that individual ants had poorer body condition and performance through time when used multiple times in the running tube.

Results

The critical-thermal-limit investigations indicated a CT_{min} of 3.3° ± 0.6°C (n = 80) and a CT_{max} of 46.4° ± 0.7°C (n = 58).

Table 1: Predictions of the orthogonal polynomial contrast analyses for the five acclimation hypotheses tested

Effect	AMR	Running Speed	COT
Beneficial acclimation	L+/−, Q+	L+/−, Q−	L+/−, Q+
Optimal acclimation	Q+	Q−	Q+
Colder-is-better	L+	L−	L+
Warmer-is-better	L−	L+	L−
No plasticity	NS	NS	NS

Note. Traits investigated were active metabolic rate (AMR, mL CO_2 h^{-1}), running speed (m s^{-1}), and cost of transport (COT, J kg^{-1} m^{-1}). L+ and L− denote positive and negative linear effects; Q+ and Q− denote positive and negative quadratic effects; NS = not significant.

In the acclimation trials, 614 measurements of running speed, AMR, and COT were obtained from a total of 135 individuals (mean body mass ± SD = 3.9 ± 1.5 mg). Body mass did not differ among ACC ($F_{2,126} = 1.090$, $P = 0.34$) or TT groups ($F_{2,126} = 0.019$, $P = 0.91$), nor was the interaction term significant ($F_{4,126} = 0.566$, $P = 0.69$).

ACC (20°, 25°, or 30°C) had no significant effect on AMR, although TT (20°, 25°, or 30°C) had a significant positive effect (Table 2; Fig. 3*A*). For AMR, interactions between ACC and TT, which might indicate beneficial acclimation, were not detected (Table 2). For running speed, both TT and ACC had significant positive effects. The quadratic effect for ACC was highly significant and concave downward (Table 3; Fig. 3*B*), suggesting strong support for the OAH. The linear effect of ACC was significantly positive ($P = 0.02$), also providing some support for the BAH, though with Figure 3*B* illustrating the major effect of optimal acclimation. For COT, no significant effects of ACC or TT were found. Therefore, the NA effect was apparent (Table 4; Fig. 3*C*).

AMR was generally repeatable at all temperatures ($r = 0.371$–0.683), and no obvious effects of thermal conditions on repeatability of AMR were observed (Fig. 4*A*). Repeatability of running speed ($r = 0.191$–0.826) was more variable than that of AMR, and temperature had significant effects on repeatability (as determined by a lack of overlap of 95% confidence limits; Fig. 4*B*). For ants acclimated at 25°C and tested at 30°C, repeatability was highest ($r = 0.826$) and significantly greater than that in ants acclimated at 25°C and tested at 25°C or

Table 2: Outputs of the ordered-factor ANOVA with single-degree-of-freedom orthogonal polynomial contrasts for the effects of acclimation and test temperature on $\log_{10}$ active metabolic rate (mL CO_2 h^{-1})

Source	df	Type III SS	MS	*F*	*P*
Acclimation temperature (ACC)	2	.0035	.0018	.10	.9045
Test temperature (TT)	**2**	**.8975**	**.4488**	**25.38**	**<.0001**
ACC × TT	4	.0415	.0104	.59	.6726
Error	112	.9620	.0175		
Contrast	**df**	**Contrast SS**	**MS**	***F***	***P***
TT-linear	**1**	**.8789**	**.8789**	**49.71**	**<.0001**
TT-quadratic	1	.0187	.0187	1.06	.3068
ACC-linear	1	.0030	.0030	.17	.6827
ACC-quadratic	1	.0006	.0006	.03	.8564
ACC × TT-linear	2	.0302	.0151	.86	.4282
ACC × TT-quadratic	2	.0113	.0056	.32	.7275
Parameter		**Estimate**	**SE**	***T***	***P***
TT-linear		**.2210**	**.0313**	**7.05**	**<.0001**
TT-quadratic		.0558	.0543	1.03	.3068
ACC-linear		−.0128	.0313	−.41	.6827
ACC-quadratic		.0098	.0543	.18	.8564
ACC × TT-linear		.0581	.0768	.76	.4508
ACC × TT-quadratic		−.0533	.1330	−.40	.6895

Note. Overall model $r^2 = 0.350$. SS = sum of squares; MS = mean squares. Significant effects are highlighted in boldface.

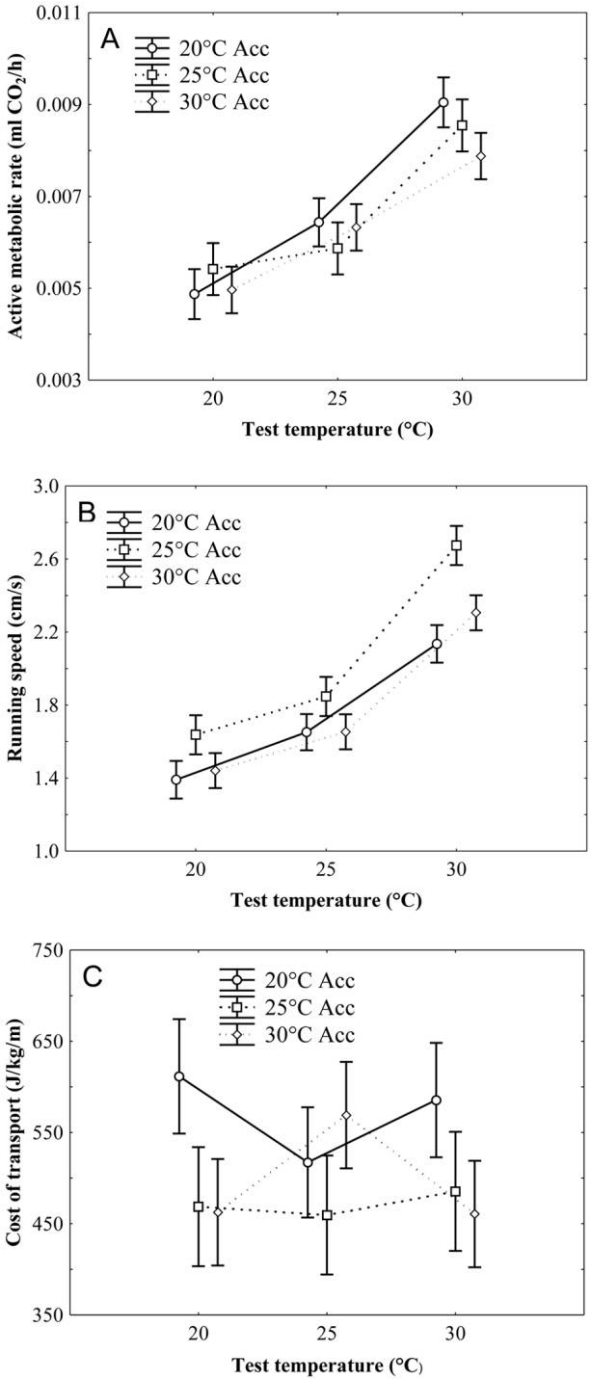

Figure 3: Mean active metabolic rate (±SE, mL CO_2 h^{-1}; *A*), mean running speed (±SE, cm s^{-1}; *B*), and mean cost of transport (±SE, J kg^{-1} m^{-1}; *C*) at each acclimation and test temperature. Acc = acclimation temperature.

acclimated at 30°C and tested at 30°C. For COT, repeatability was highly variable among temperatures and ranged from showing highly significant repeatability in ants acclimated and tested at 20°C ($r = 0.903$) to low repeatability for ants acclimated at 25°C and tested at 20°C and 25°C ($r = 0.215$ and 0.166, respectively; Fig. 4*C*).

Discussion

Using a strong-inference approach to distinguish among five working hypotheses (Table 1; Huey et al. 1999), this study revealed that in the seed-harvester ant *Messor capensis*, both AMR and COT show no plasticity, whereas running speed showed evidence thereof. Indeed, individuals acclimated at 25°C ran consistently faster at all TTs than those acclimated at 20° and 30°C, providing support for the OAH (Cohet and David 1978). Given the limited information on phenotypic plasticity of locomotion performance in insects (see "Introduction"; Irschick and Garland 2001), this study highlights the importance of measuring several traits when assessing short- and long-term effects of temperature on locomotion performance.

Despite much variability in the findings of other thermal-acclimation studies (reviewed in Angilletta 2009), the support for the OAH in *M. capensis* is in keeping with other investigations of plasticity of insect walking speed (Crill et al. 1996; Gibert et al. 2001) and of various performance traits (e.g., Cohet and David 1978; Huey et al. 1995, 1999; Zamudio et al. 1995). This result was clearly not an artifact of stressful ACCs (see Huey et al. 1999), given that these temperatures were well within the critical limits of the species (ca. 16°C from either limit) and well within the range of temperatures likely to be encountered by ants foraging in the field (Fig. 1).

Whether support for the OAH indicates an adaptive response is more difficult to ascertain, because a direct link between locomotion performance and fitness benefits is not always straightforward to establish and because such links have been generally poorly explored (Irschick and Garland 2001; Irschick et al. 2008). Nonetheless, given the significance of foraging to ants generally and seed harvesters in particular (Lighton et al. 1993), a link between aspects of locomotion performance and fitness seems likely. Indeed, in this case specifically, the lack of acclimation response of AMR and COT but the increase in speed after acclimation to 25°C may well represent some advantage, because reduced costs of locomotion will enhance energy availability for other purposes (for a general discussion, see Clarke 2003). Why AMR and COT showed NA effects is not clear, but the absence of a response across optimal temperatures has previously been found in ectotherms (see Cossins and Bowler 1987). Given that standard metabolic rate often shows considerable change after acclimation (see, e.g., Terblanche et al. 2009 and discussion in Chown and Gaston 1999), further work across a range of species is needed to determine whether AMRs are less responsive than standard metabolic rates to acclimation.

The benefits of acclimation for locomotion performance may also be apparent only for specific performance traits (Kingsolver and Huey 1998). For example, while CT_{min} readily responds to acclimation (e.g., in ants; Jumbam et al. 2008), shifts in optimal temperature are less prominent (Chown and Nicolson 2004; Angilletta 2009), and CT_{max} tends to show much less variation (Chown 2001) and may be limited by low heritability (Mitchell and Hoffmann 2009). Nonetheless, the current evidence in-

Table 3: Outputs of the ordered-factor ANOVA with single-degree-of-freedom orthogonal polynomial contrasts for the effects of acclimation and test temperature on $\log_{10}$ running speed (m s^{-1})

Source	df	Type III SS	MS	*F*	*P*
Acclimation temperature (ACC)	**2**	**.1891**	**.0946**	**7.95**	**<.001**
Test temperature (TT)	**2**	**.7838**	**.3919**	**32.93**	**<.0001**
ACC × TT	4	.0104	.0026	.22	.9270
Error	112	1.4408	.0129		
Contrast	df	Contrast SS	MS	*F*	*P*
TT-linear	**1**	**.7653**	**.7653**	**64.31**	**<.0001**
TT-quadratic	1	.0185	.0185	1.56	.2148
ACC-linear	**1**	**.0667**	**.0667**	**5.61**	**.0198**
ACC-quadratic	**1**	**.1224**	**.1224**	**10.28**	**.0018**
ACC × TT-linear	2	.0011	.0006	.05	.9531
ACC × TT-quadratic	2	.0093	.0046	.39	.6776
Parameter		Estimate	SE	*T*	*P*
TT-linear		**.2062**	**.0257**	**8.02**	**<.0001**
TT-quadratic		.0556	.0445	1.25	.2148
ACC-linear		**.0609**	**.0257**	**2.37**	**.0198**
ACC-quadratic		**−.1428**	**.0445**	**−3.21**	**.0018**
ACC × TT-linear		−.0192	.0630	−.31	.7606
ACC × TT-quadratic		−.0571	.1091	−.52	.6018

Note. Overall model $r^2 = 0.455$. SS = sum of squares; MS = mean squares. Significant effects are highlighted in boldface.

Table 4: Outputs of the ordered-factor ANOVA with single-degree-of-freedom orthogonal polynomial contrasts for the effects of acclimation and test temperature on the cost of transport (J kg^{-1} m^{-1})

Source	df	Type III SS	MS	*F*	*P*
Acclimation temperature (ACC)	2	285,517.71	142,758.85	2.63	.0772
Test temperature (TT)	2	6,929.14	3,464.57	.06	.9382
ACC × TT	4	125,368.91	31,342.23	.58	.6799
Error	112	5,725,399	51,120		
Contrast	df	Contrast SS	MS	*F*	*P*
TT-linear	1	6,589.40	6,589.40	.12	.7283
TT-quadratic	1	339.74	339.74	.01	.9371
ACC-linear	1	210,121.45	210,121.45	3.87	.0520
ACC-quadratic	1	75,396.26	75,396.26	1.39	.2415
ACC × TT-linear	2	19,188.77	9,594.39	.18	.8383
ACC × TT-quadratic	2	106,180.14	53,090.07	.98	.3799
Parameter		Estimate	SE	*T*	*P*
TT-linear		−19.13	54.93	−.35	.7283
TT-quadratic		7.52	95.14	.08	.9371
ACC-linear		−108.04	54.93	−1.97	.0520
ACC-quadratic		112.10	95.14	1.18	.2415
ACC × TT-linear		−79.03	134.55	−.59	.5583
ACC × TT-quadratic		119.80	233.05	.51	.6084

Note. Overall model $r^2 = 0.072$. SS = sum of squares; MS = mean squares.

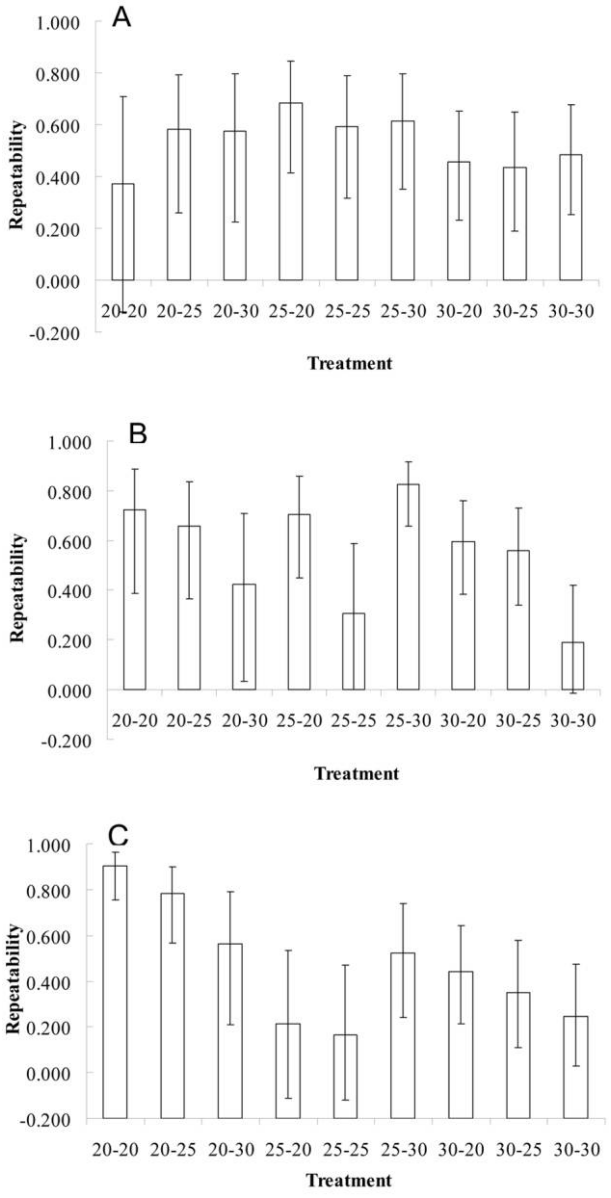

Figure 4: Repeatability (±95% confidence levels) of active metabolic rate (mL CO_2 h^{-1}; *A*), voluntary running speed (m s^{-1}; *B*), and cost of transport (J kg^{-1} m^{-1}; *C*) for each combination of acclimation (ACC) and test (TT) temperature (first number given in axes legend is ACC, the second number is TT).

dicates that for running speed, the BAH is not supported, and the maximization of speed and minimization of the costs thereof may have resulted in differences in response among traits. This outcome supports Angilletta's (2009) contention that understanding the likely fitness advantages of particular responses may be more significant than trying to draw distinctions between the particular forms of these responses.

The outcome of the repeatability estimates for these performance traits is also noteworthy. Most studies of repeatability in physiological traits of insects are conducted under a very specific set of conditions, and usually temperature is held constant (e.g., resting metabolic rate; Nespolo et al. 2003, 2007; Terblanche et al. 2004*a*, 2004*b*; Chown et al. 2006). However, few studies report repeatability of a particular trait under a range of experimental conditions (but see, e.g., Deere and Chown 2006; Clusella-Trullas et al. 2007 for other noninsect groups). While significant repeatability of locomotor performance has previously been demonstrated in other taxa (e.g., Bennett 1980; Huey and Dunham 1987; van Berkum et al. 1989; Huey et al. 1990; Kolok 1999; Deere and Chown 2006; Outfiero and Garland 2009), to our knowledge, none have demonstrated short-timescale repeatability of running speed in terrestrial in-

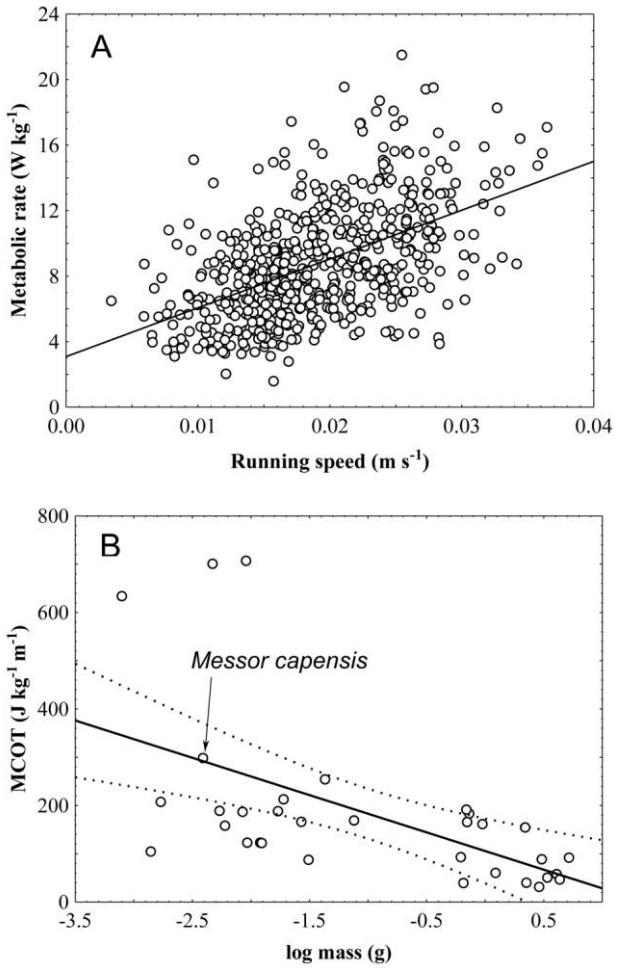

Figure 5: *A*, Scatter plot of mass-specific metabolic rate against voluntary running speed from all trials ($n = 614$) of *Messor capensis* ($y = 298.06x + 3.0869$; $r^2 = 0.2731$, $P < 0.0001$). *B*, Insect allometric scaling of minimum cost of transport (MCOT) derived from data presented by Fleming and Bateman (2007) and additional data compiled from the literature (Weier et al. 1995; Berrigan and Partridge 1997). Adult MCOT measured at 25°C was selected when multiple data were available for the same species. Values were converted to J s^{-1} kg^{-1}, assuming a respiratory quotient of 0.8 and energy conversion of 23.3 J mL CO_2^{-1} (for consistency with the methods of Fleming and Bateman 2007). The interspecific relationship is MCOT = 105.7 − 77.3 × log body mass ($r^2 = 0.31$, $F_{1,31} = 13.67$, $P < 0.001$). Dashed lines represent 95% confidence limits.

sects across several temperature and acclimation treatments. One striking result from these analyses is that each of the traits examined responded in different ways when assayed across varying TTs and ACCs. For example, AMR was relatively high and fairly consistently repeatable, with r values in the range of those previously published for resting metabolic rate (reviewed in Nespolo and Franco 2007). Running speed appeared less repeatable when tested at the same temperature as the ACC, at least for the 25° and 30°C groups. By contrast, for *M. capensis* acclimated at 20°C, running speed was highly repeatable at a TT of 20°C and less repeatable at other TTs. This latter result suggests that some form of thermal constraint might result in more consistent performance among *M. capensis* individuals under cooler conditions. On the other hand, at warmer temperatures, the insects are not constrained in their performance, thus possibly allowing greater intraindividual variability and hence lower repeatability. In addition, the repeatability results of this study show that an important prerequisite of natural selection—namely, consistent among-individual variation—is generally met for running speed, AMR, and COT but that the ACC-TT treatment combination used influences the outcomes of the intraindividual and interindividual variability components (for similar discussions, see Hayes et al. 1998; Chown et al. 2009). Whether these patterns remain or become more stable at longer timescales (i.e., longer-term repeatability such as across days and months) should be explored further. Here, repeatability was examined over intervals of 10–60 min, whereas most other studies repeat measures at intervals of several days (e.g., Marais and Chown 2003; and see Jayne and Bennett 1990; Austin and Shaffer 1992; Outfiero and Garland 2009 for several timescale effects).

Estimation of the MCOT (the slope of the relation between walking speed and metabolic rate) enables the COT of *M. capensis* to be compared with that found for insects more generally (see Berrigan and Lighton 1993; Fleming and Bateman 2007). Following Lighton et al. (1993) and Lighton and Duncan (2002), individual MCOT values were homogeneous within each ACC-TT group (ANCOVA, $P > 0.28$ in all nine cases), and MCOT was statistically homogeneous in a full ANCOVA model that assessed the influence of ACC, TT, and their potential interactions on the homogeneity of slopes (ANCOVA, $P > 0.11$). Therefore, MCOT values were pooled across all data to derive an estimate of MCOT of 298 J kg^{-1} m^{-1} for *M. capensis* (Fig. 5*A*). This value is comparable to the MCOT calculated from ACC and TT at 25°C, 296 J kg^{-1} m^{-1}. Either way, the MCOT of *M. capensis* (average mass = 3.9 ± 1.5 mg) is well within the range expected for insects of similar size (see Fig. 5*B*). Clearly, however, there is considerable variation in MCOT among species, and methodological approaches also have an influence (discussed in Chown and Nicolson 2004). For example, some ant species (e.g., 5-mg *Formica* spp.; Jensen and Holm-Jensen 1980) have a considerably higher MCOT, ~700 J kg^{-1} m^{-1}, and there is much debate about the appropriate method for estimating MCOT (see, e.g., discussions in Lighton et al. 1993).

In conclusion, this study suggests an important role for long- and short-term temperature effects on locomotion speed, AMR, and COT in ants. In particular, the occurrence of clear support for the OAH for running speed but NA effects for the other two traits emphasizes the need to investigate a variety of traits relevant to performance. While calls for additional work in an area can almost always be made, it is noteworthy that so few studies have examined acclimation effects on locomotion and its energetic costs in insects. Given their prominence among ectotherms, to reach a conclusion about the scope and relative benefits of acclimation in these traits might be premature, despite much work on vertebrate ectotherms.

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